

Characterization of *Pfiesteria* Ichthyocidal Activity

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Article:

Drgon et al. (4) concluded that the “aquarium bioassay format is unsuitable to accurately assess the ichthyocidal activity of *Pfiesteria* spp.” and “ichthyocidal activity of *Pfiesteria* spp. is mostly due to direct interactions of the zoospores with fish skin and gill epithelia rather than to soluble factors.” These conclusions are not justified, because microbial community analyses of control aquariums were not included and previous studies (5, 8) that utilized similar experimental approaches and found significant (100%) fish death attributable to soluble factors were overlooked.

It is known a priori that aquariums containing fish or inoculated with whole sediment will develop a complex microbial community. This has been previously noted for the aquarium bioassay for *Pfiesteria* ichthyotoxicity (2). Given this complexity, a key factor in attributing fish death to *Pfiesteria* spp. is the difference between *Pfiesteria*- or sediment-inoculated aquariums and corresponding controls.

Drgon et al. focused on the presence of potentially pathogenic *Vibrio* spp. in experimental aquariums as a problem with attributing fish death to *Pfiesteria* spp. A recently published study of which the authors would not have been aware (1) showed that *Vibrio* spp. can be more abundant in control aquariums where fish remain healthy, and a previous publication showed that total bacterial numbers do not differ significantly between experimental aquariums with *Pfiesteria* spp. and control aquariums (7). Denaturing gradient gel electrophoresis of bacterial assemblages from control and experimental aquariums has shown that there is no consistent difference in community composition between experimental and control aquariums (1). These studies illustrate the importance of comparison between microbial communities in aquariums inoculated with clonal *Pfiesteria* cultures or sediment and corresponding controls. The organisms that toxic aquariums consistently have in common and that are absent from controls are *Pfiesteria* spp., implicating *Pfiesteria* spp. in fish death.

Drgon et al. concluded that most of the fish death in aquariums containing *Pfiesteria* spp. requires direct contact between fish and *Pfiesteria* spp. While this was true for their experiment and is consistent with another recent study that utilized similar methods (6), the authors failed to cite earlier publications that showed up to 100% death of finfish in cell-free filtrates from toxic *Pfiesteria* cultures (5) and 100% death of bivalve larvae in containers where toxic *Pfiesteria* spp. were held in dialysis tubing (molecular mass cutoff, 12 to 14 kDa) (5). Their results should have been discussed in the context of these previous studies using different *Pfiesteria* strains, where mortality was mostly attributable to soluble factors.

In summary, the article by Drgon et al. is flawed, since data on microbial communities in controls corresponding to sediment- or *Pfiesteria*-inoculated aquariums were not included. Thus, no conclusion regarding the suitability of the aquarium bioassay format can be made from their study. The authors also failed to acknowledge previous publications that showed high mortality in cell-free filtrates from toxic *Pfiesteria* sp. cultures. When the available data are objectively considered, the conclusion that emerges is that toxicity by *Pfiesteria* spp. is mediated by both direct contact and soluble toxic factors. The relative contribution of each

depends on the *Pfiesteria* strains studied, culture history, and methods utilized to detect soluble toxins (1, 3).

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